Supplement to DER for MRID [Accession No. ~~259442-259446~~]- 2-Generation Reproduction Study - Rat [2,4-D] MRID 00150557/00163996

This supplement provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document Nos. 005446, 005684, 005754].

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AMENDED DATA EVALUATION RECORD

STUDY TYPE: 2-generation reproduction - rat § 83-4/OPPTS 870.3800
P.C. CODE: 030001
CASWELL NO.: 315 CAS #: 94-75-7
TEST MATERIAL (PURITY): 2,4-D [97.5% a.i.]
SYNONYMS: 2,4-dichlorophenoxyacetic acid
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Kopp, S., Leist, P. L., Mercieca, M. D., *et al.*. (1985). A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid. Study No. WIL-81137, July 26, 1985. Accession Nos. ~~259442-259446~~]. Document No. 005446 and Addendum: Accession No. 265489, Document Nos. 005754 and 005684. Unpublished. MRID 00150557 and MRID 00163996

MRID 00150557 & 00163996

EXECUTIVE SUMMARY: In a 2-generation reproduction study [MRID (Accession No. 259442-259446,265489)], 30 male/30 female F0 Fischer 344 rats/sex/group were administered 2,4-D [97.5% a.i.] *via* the diet for 105 days prior to mating and through gestation and lactation of two litters and for 30 days after weaning the last litter at target dose levels of 0, 5, 20, and 80 mg/kg/day. Rats were mated, one male with one female. The resulting F1a litters were weaned at day 28 *post partum*. After a 2-week rest period, the F0 parental rats were re-bred using different male/female combinations to produce the F1b litters, from which 30 males/30 females/group were selected to become the F1 parents. The F1 generation [30 rats/sex/group] was administered the test material at target dose levels of 0, 5, and 20 mg/kg/day [**high-dose level dropped due to excess toxicity**; there were an insufficient number of F1b pups] *in utero* and continuously *via* the milk or feed for 125 days postnatally and prior to mating and through gestation and lactation of two litters [F2a and F2b] and for 30 days after weaning the last litter.

There were no apparent treatment-related deaths, and clinical signs were comparable among the groups throughout the study. During the *pre-mating dosing period*, body weights of the **F0 parental animals** were slightly lower [males 95%-97% (by week 6)/females 95%-96% (by week 13) of control] at the high-dose level for both sexes. Body-weight gains of the F0 high-dose males were decreased initially [weeks 2-3 (86% of control] and overall [weeks 0-13 and weeks 0-40 (93% of control], as were those of the high-dose females [weeks 0-1 (79% of control); weeks 0-13 (92% of control) and weeks 0-40 (94% of control)] compared to the controls.

The high-dose **F0 dams** displayed a significantly lower body weight throughout [F1A litter] gestation (94%-95% of control) and by gestation day 20 during F1b pregnancy [90% of control]. The high-dose F0 dams displayed significantly reduced body-weight gains compared to the controls during both gestation periods, with the greater

deficit being observed during the second gestation period [F1a litters: days 0-7 (67%* of control); days 13-20 (95% of control; days 0-20 (87% of control); F1b litters: days 0-7 (70%* of control); days 13-20 (59%** of control); days 0-20 (67%** of control)]. The high-dose F0 dams displayed decreased body weight on day 7 of lactation [both litters; 92%-93% of control], but body weights were significantly increased compared to the controls at day 28 of lactation [F1a (108%/F1b 111% of control]. Body-weight gains were significantly reduced during lactation days 1-7 for both litters [F1a (40% of control); F1b (6% of control)]. Overall, however, the high-dose dams displayed positive body-weight gain during lactation days 1-28 compared to negative body-weight gains in the control and other dose groups.

Food consumption [g/rat/day] during the pre-mating period was slightly lower [94%-95% of control] in the high-dose females during a few weeks, but on a g/kg/day basis, both sexes at the high-dose level displayed a slight increase [104% of control] in food consumption compared to the controls. During the first week of the two-week rest period following the weaning of the first litter, the F0 dams displayed a significant decrease in food consumption [83%-84% of control]. Food consumption was decreased at the high-dose level during both gestation periods [F1a during first 2 weeks (91%-93% of control); F1b during third week (82% of control)]. A significant decrease in food consumption was observed throughout lactation [both litters] at the high-dose level [F1a litter (58% of control for days 1-28); F1b litter (71%-83% of control)]. At necropsy, no treatment-related adverse effects were observed at any dose level, although the F0 females displayed increased kidney weights at all dose levels but there was no dose response.

There were no apparent, treatment-related, adverse effects on body weights or body-weight gains of the **F1 parental animals** during the pre-mating dosing period at the two remaining dose levels, although the mid-dose [20 mg/kg/day; the highest dose in the F1 generation] males displayed an initial decrease in body-weight gain [weeks 35-36 (91%** of control) and weeks 36-37 (89%** of control)]. At 20 mg/kg/day, there were no significant differences in body weights in the **F1 dams** during gestation [F2a litters 95%-99%; F2b litters 95%-96% of control] or body-weight gains F2a litters 85% (days 7-13); F2b litters 83% (days 0-7); 86% (days 13-20); 90% (days 0-20) of control], and comparable body weights/gains were observed during lactation [both litters]. Food consumption was comparable among the groups [both sexes] throughout the study. At necropsy, no treatment-related adverse effects were observed at either dose level, although the F1 males and females displayed slightly increased kidney weights at the 20 mg/kg/day dose level, and the females at this dose level displayed a slight increase in liver weight.

F0 Generation. No apparent adverse effect was observed on fertility. Pre-coital intervals were comparable among the groups. The duration of gestation was significantly increased in the high-dose [80 mg/kg/day] F0 females producing the F1b pups [22.5 days *vs* 21.9 days]. The gestation survival index was comparable among the groups for the F1a pups but significantly decreased for the F1b litters [31.7% *vs* 97.8%]. There was a significant decrease in the number of F1a female fetuses at the high-dose level [39% *vs*54%]. The number of F1b pups born dead/dying by day 1 [110] was significantly increased at the high-dose level compared to the control [5]. F1a litter size was slightly lower at the high-dose level compared to the control [9.0 *vs* 10.1], but F1b litter size was significantly lower than the control [5.1** *vs* 9.5]. F1a pup viability was comparable throughout weaning, but F1b pup viability was significantly lower throughout the weaning period. There was a significant decrease in F1b pup survival to lactation day 4 at the high-dose level [86.3%] compared to the control [100%] and other dose levels [98% and 99.6%], as well as survival to lactation day 28 [71.4% *vs* 100% (control) and other dose groups 99.4% and 100%]. Decreased pup body weight [F1a males 89%/females 90% of control (day 1), 75%/81% of control (day 28); F1b males 78%/females 85% of control (day 1), 73%/76% of control (day 28)] and body-weight gains [F1a males 68%/females 70% of control (days 1-4), 75%/80% of control (days 4-28); F1b males 26%/females 43% of control (days 1-4), 76%/78% of control (days 4-28)] were observed at the high-dose level, with the F1b litters displaying the greater

effect. At the mid-dose level, there was a slight decrease in body weight [F1a males93%/females 94% of control (day 28); F1b males 84%/females 87% of control (day 28)] and body-weight gains [F1a males 92%/females 93% of control (days 4-28); F1b males 83%/females 85% of control (days 4-28)], with the deficits being greater in the F1b litters.

Skeletal anomalies and reduced ossification were observed in the high-dose F1b pups [80 mg/kg/day] that were dead at birth [only dose level examined].

F1 Generation. No apparent adverse effect was observed on fertility at either dose level. Pre-coital intervals and gestation lengths were comparable among the groups. The gestation survival index and the viability index were comparable among the groups for both the F2a and F2b litters. Litter size, body weights, and the sex ratio were comparable among the groups in both the F2a and F2b litters.

Degenerative changes in the tubules of the cortical region [high-dose F0 males] and outer medullary regions [mid- and high-dose F0 males, mid-dose F1 males (highest dose tested in this generation)] of the kidneys were found in a subsequent histopathological examination. The original reviewer noted that these effects on the kidney were not found originally but during a subsequent re-examination of the tissues, casting doubt on the quality of the histopathological examination of the reproductive organs. However, the RfD/QA Peer Review Committee determined that, based on the lack of effects on reproductive organs in the chronic and subchronic studies at similar or higher dose levels, reevaluation of these tissues [testes and ovaries] is not necessary [HED Document No. 011908, dated 5/9/96].

The NOAEL for parental toxicity is 5 mg/kg/day (target dose; actual dose range 3.8-13.5 mg/kg/day) and the parental LOAEL is 20 mg/kg/day (target dose; actual dose range 14-48 mg/kg/day), based on decreased female body weight/body- weight gain (F1) and male renal tubule alteration (F0 and F1).

The NOAEL for reproductive toxicity is 20 mg/kg/day (target dose; actual dose range 18-35 mg/kg/day), and the LOAEL for reproductive toxicity is 80 mg/kg/day (target dose; actual dose range 69-114 mg/kg/day), based on an increase in gestation length.

The NOAEL for offspring toxicity is 5 mg/kg/day (target dose; actual dose range 7.2-13.5 mg/kg/day), and the LOAEL for offspring toxicity is 20 mg/kg/day (target dose; actual dose range 26-48 mg/kg/day), based on decreased pup body weight [F1b]. At 80 mg/kg/day (target dose; actual dose range 76.1-133 mg/kg/day), there was an increase in pup deaths.

This 2-generation reproduction study is classified Acceptable/guideline,. This study satisfies the guideline requirement (OPPTS 870.3800; §83-4) for a 2-generation reproduction study.

Table 1. Body-Weight/Gains [grams] During Premating Period				
Generation/Sex/Week/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 MALES [body weight (g)]				
week 0	113.4±7.6	114.2±7.4	112.3±6.0	111.2±6.4
week 1	149.9±7.5	151.4±10.1	148.3±7.8	146.0±8.4
week6	261.0±9.3	262.5±12.3	258.9±11.2	253.7±10.4* [97]♪
week 10	299.4±10.3	299.7±12.5	292.6±12.9	286.2±10.8*
week 13	316.3±11.7	313.8±14.7	310.1±14.5	299.0±12.7** [95]
week 40	372.4±15.4	373.2±17.8	368.0±18.3	353.1±19.5** [95]
F0 FEMALES [body weight (g)]				
week 0	90.4±4.7	91.2±4.6	91.3±4.5	90.8±4.7
week 1	110.2±5.4	110.5±5.2	108.8±4.8	106.4±5.2
week6	158.5±7.6	161.8±7.9	159.3±7.1	155.4±7.8
week 10	173.3±8.3	175.3±8.6	174.0±7.7	168.1±9.2
week 13	179.0±9.1	179.6±9.5	180.2±8.3	172.7±9.7** [96]
week 40	216.2±10.9	219.3±8.6	214.8±7.7	206.2±12.1** [95]
F1 MALES [body weight (g)]				
week 35	112.5±18.8	123.5±19.4	122.2±14.6 [109]	-
week 36	151.4±18.3	158.6±21.5	157.4±15.0 [104]	-
week 37	191.2±17.0	192.6±21.0	192.7±14.2 [101]	-
week 49	331.0±12.6	326.1±22.9	323.8±18.2 [98]	-
week 65	374.2±13.2	370.1±24.9	364.4±19.5 [97]	-
week 77	394.2±13.7	398.1±26.2	384.4±22.5 [98]	-
F1 FEMALES [body weight (g)]				
week 35	99.6±16.5	100.8±10.7	94.1±11.2 [94]	-
week 36	117.4±16.4	117.8±9.1	115.1±9.6	-
week 37	131.9±17.1	130.8±8.0	130.6±9.9	-
week 53	204.1±9.0	200.7±9.3	198.1±10.4	-
week 77	237.9±9.5	233.1±8.2	231.7±10.2* [97]	-
F0 MALES [body-weight gain (g)]☹				
weeks 0-1	36.5	37.2	36.0	34.8 [95]
weeks 1-2	33.6	35.8*	36.0*	36.2**
weeks 2-3	26.5	24.5* [93]	25.5	22.9** [86]
weeks 0-13	202.9	199.6 [98]	197.8 [97]	187.8** [93]
weeks 0-40	259.0	259.0	255.7	242.0** [93]
F0 FEMALES [body-weight gain (g)]☹				
weeks 0-1	19.8	19.3	17.4** [88]	15.6** [79]
weeks 2-3	10.6	11.2	10.9	9.7 [92]
weeks 0-13	88.6	88.4	88.9	81.9** [92]
weeks 0-40	122.8	128.1	123.5	115.4** [94]
F1 MALES [body-weight gain (g)]				
weeks 35-36♬	38.9±5.1	35.1±4.7** [90]	35.2±4.3** [91]	-
weeks 36-37♬	39.7±5.0	34.0±5.6** [86]	35.3±3.9** [89]	-
weeks 35-75 ✖	274.5	258.5 [94]	254.6 [93]	-
weeks 35-77 ✖	281.7	274.6 [97]	262.2 [93]	-
F1 FEMALES [body-weight gain (g)]				
weeks 35-36♬	17.9±4.6	16.9±3.1	21.0±5.5* [117]	-
weeks 36-37♬	14.4±3.1	13.1±3.2	15.5±2.8 [108]	-

♪ [% of control]; ☹ data from Tables 2-4 (pages 61-70 of Volume 1; study report did not provide s.d.); ✖ calculated by this reviewer (no statistics performed); data from volume 1, pages 61-72, Appendix B, pages 162-227; from Tables 2 & 3, pages 737-767 of Volume 4 of study report]; ♬ from Table 3, pages 35 and 44 of Volume 4]; * $p<0.05$; ** $p<0.01$;

Table 2. Food Consumption ☹ During Mating Period, Gestation, and Lactation - F0 Generation								
Generation/Sex/Week/Dose	0 mg/kg/day		5 mg/kg/day		20 mg/kg/day		80 mg/kg/day	
F0 MALES [g/rat/day]								
week 1	14.5		14.7		14.8		14.2	
week 3	17.6		17.6		17.8		17.2	
week 12	16.5		16.8		16.7		16.0 [97]♪	
week 15	15.7		16.1		16.9**		15.3	
week 24	16.5		16.5		16.5		15.9* [96]	
week 25	16.3		16.6		16.5		16.2	
F0 FEMALES [g/rat/day]								
week 1	11.2		12.0*		11.7		11.5	
week 3	12.5		12.6		12.2		11.8 [94]	
week 12	11.3		11.7		11.7		10.7** [95]	
week 15	10.7		10.9		11.2		10.7	
week 24	16.7		16.9		15.8 [95]		13.9** [83]	
week 25	14.6		15.0		14.4		14.0 [96]	
F0 MALES [g/kg/day]								
week 1	110.5		110.5		113.3*		110.6	
week 3	89.2		88.4		90.4		88.8	
week 12	53.6		54.9*		55.7**		54.8	
week 15	50.2		51.8*		54.9**		51.5	
week 24	48.7		48.7		49.3		49.3	
week 25	47.7		48.8		49.0*		49.8**	
F0 FEMALES [g/kg/day]								
week 1	112.1		118.8**		116.8		116.5	
week 3	96.3		96.1		93.3		92.6	
week 12	64.4		65.6		66.2		63.0	
week 15	60.6		68.4**		62.9		63.1	
week 24	86.0		61.6		80.6		72.6** [84]	
week 25	72.7		74.2		71.7		72.2	
F0 FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
gestation F1A mating								
days 0-7	11	61	11	61	11	60	10* [91]	59
days 7-13	14	68	13	66	13	68	13** [93]	68
days 13-20	14	63	13	65	13	65	14	65
gestation F1B mating								
days 0-7	13		13		13		12	
days 7-13	14		15		16*		14	
days 13-20	17		17		17		14** [82]	
F0 FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
lactation F1A mating								
days 1-7	23	118	24	118	22	115	18** [78]	96** [81]
days 7-14	36	172	36	172	34	167	29** [81]	144** [84]
days 14-21	44	204	43	204	44	205	39* [89]	186 [91]
days 21-28	59	291	54	273	55	273	45** [76]	213** [73]
days 1-28		203		199		197		169** [58]
lactation F1B mating								
days 1-7	24	109	26	116	25	116	16** [67]	77** [71]
days 7-14	36	160	38	162	38	168	27** [75]	124** [78]
days 14-21	44	191	45	189	45	193	36* [82]	158* [83]
days 21-28	55	256	52	239	51	238	45** [82]	196** [77]

♪ [% of control]; ☹ data from Tables 13-22 (pages 81-97 of the report; study report did not provide s.d.); * p<0.05; ** p<0.01

Table 3. Food Consumption ☹ During Gestation and Lactation - F1 Generation								
Generation/Sex/Week/Dose	0 mg/kg/day		5 mg/kg/day		20 mg/kg/day		80 mg/kg/day	
F♀ FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
gestation F2A mating								
days 0-7	10	48	10	49	11*	56*	-	-
days 7-13	13	60	13	62	14	64	-	-
days 13-20	16	64	16	66	16	65	-	-
days 0-20	13	57	13	59	14	61**	-	-
gestation F2B mating								
days 0-7	12	51	12	53	11	52	-	-
days 7-13	15	62	16	65	15	66	-	-
days 13-20	18	65	17	64	16	61	-	-
days 0-20	15	59	15	60	14	59	-	-
F♀ FEMALES	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day	g/rat/day	g/kg/day
lactation F2A mating								
days 1-7	25	111	23	106	24	111	-	-
days 7-14	37	161	34	152	37	161	-	-
days 14-21	45	194	41	177	43	184	-	-
days 21-28	62	273	61	271	65	285	-	-
days 1-28	43	189	41	182	43	191	-	-
lactation F2B mating								
days 1-7	24	99	25	103	24	104	-	-
days 7-14	37	147	37	153	36	150	-	-
days 14-21	47	181	49	196	48	192	-	-
days 21-28	64	267	64	270	65	276	-	-
days 1-28	44	179	44	185	44	186	-	-

♪ [% of control]; ☹ data from Tables 12-21 (pages 779-804 of the report; study report did not provide s.d.); * p<0.05; ** p<0.01

Table 4. Female Body Weight [grams]✖ During Gestation				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] Gestation days				
0	178±7	179±9	178±8	173±9
7	190±9	191±10	191±9	181±10** [95]
13	208±9	208±10	206±8	196±10** [94]
20	246±21	252±18	249±11	232±16* [94]
F0 [F1b litter] Gestation days				
0	200±11	205±10	202±9	197±11
7	210±11	214±9	210±9	204±12
13	226±12	232±11	230±9	218±13 [96]
20	270±20	277±21	274±18	244±17** [90]
F1 [F2a litter] Gestation days				
0	201±7	198±8	198±12	-
7	221±17	208±12	211±13	-
13	234±9	227±9	228±14	-
20	271±24	271±17	270±17	-
F1 [F2b litter] Gestation days				
0	222±10	221±10	214±11	-
7	234±11	229±8	224±12	-
13	250±13	248±10	241±14 [96]	-
20	293±18	290±18	278±26 [95]	-

♪ [% of control]; ✖ data from Appendices C (pages186-192) and E (pages 200-205) of Volume 1 and Appendices C (pages 930-934) and E (pages 940-944) of Volume 4 of the study report; * p<0.05; ** p<0.01;

Table 5. Female Body-Weight Gain [grams]✖ During Gestation				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] Gestation days				
0-7	12±4	12±4	13±4	8±5* [67]
7-13	18±5	18±6	15±5 [83]♪	15±4 [83]
13-20	38±19	44±13	44±9	35±11 [95]
0-20	68±20	74±16	71±9	59±13 [87]
F0 [F1b litter] Gestation days				
0-7	10±5	9±4	8±5	6±4* [70]
7-13	16±4	18±5	20±4	14±6 [88]
13-20	43±16	45±15	44±14	27±9** [59]
0-20	70±15	72±14	72±13	47±10** [67]
F1 [F2a litter] Gestation days				
0-7	10±15	10±13	14±5	-
7-13	20±5	19±5	17±6	-
13-20	38±20	44±13	42±10	-
0-20	70±22	73±16	72±12	-
F1 [F2b litter] Gestation days				
0-7	12±6	8±5	10±7	-
7-13	16±5	19±6	17	-
13-20	43±14	42±13	37±20 [86]	-
0-20	70±16	69±15	64±20 [90]	-

♪ [% of control]; * p<0.05; ** p<0.01; ✖ data from Appendices D (pages 193-199) and F (pages 206-211) of Volume 1 and Appendices D (pages 935-939) and F (pages 945-949) of Volume 4 of the study report

Table 6. Female Body Weight [grams]✖ During Lactation				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] lactation days				
1	189±12	191±9	191±9	184±10
7	205±10	207±11	201±9	189±10** [92]♪
14	212±12	212±17	207±17	208±10
21	216±14	213±16	219±13	212±11
28	189±14	184±14	185±18	204±15** [108]
F0 [F1b litter] lactation days				
1	210±11	215±9	208±10	205±13
7	226±15	233±12	225±11	211±14* [93]
14	228±16	237±10	233±16	224±18
21	229±19	239±15	234±19	231±12
28	203±22	197±14	193±16	226±13* [111]
F1 [F2a litter] lactation days				
1	216±8	211±6	211±13	-
7	228±11	221±9	222±16	-
14	234±23	232±10	233±16	-
21	232±16	233±7	236±16	-
28	220±16	221±12	223±18	-
F1 [F2b litter] lactation days				
1	236±11	234±9	227±13	-
7	248±14	245±8	237±18	-
14	260±13	245±16	248±17	-
21	255±8	252±12	250±15	-
28	228±16	221±11	222±12	-

♪ [% of control]; * p<0.05; ** p<0.01 ✖ data from Appendices G (pages 212-215) and I (pages 220-223) of Volume 1and Appendices G (pages 950-952) and I (pages 956-958) of Volume 4 of the study report

Table 7. Female Body-Weight Gain [grams]✖ During Lactation				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F0 [F1a litter] lactation days				
1-7	15±7	16±7	11±5	6±6** [40]♪
7-14	8±10	5±14	6±15	19±5** [238]
14-21	4±12	1±19	11±18	4±4
21-28	-27±19	-29±26	-33±20	-9±16*
1-28	-1±15	-7±18	-5±17	20±15 **
F0 [F1b litter] lactation days				
1-7	16±7	17±8	17±7	1±5** [6]
7-14	2±14	4±14	8±13	14±5
14-21	1±19	2±12	0±25	8±8
21-28	-26±19	-41±20*	-40±18*	-5±9
1-28	-7±18	-18±16	-15±17	23±4**
F1 [F2a litter] lactation days				
1-7	12±7	10±9	11±8	-
7-14	6±19	11±9	11±9	-
14-21	-2±13	1±9	3±8	-
21-28	-12±17	-12±11	-13±11	-
1-28	4±15	10±10	12±10	-
F1 [F2b litter] lactation days				
1-7	12±10	11±6	10±9	-
7-14	12±17	0±13	11±20	-
14-21	-5±16	7±17	3±14	-
21-28	-27±13	-31±13	-29±15	-
1-28	-9±10	-14±11	-5±16	-

♪ [% of control]; ✖ data from Appendices H (pages 216-219) and J (pages 224-227) of Volume 1
Appendices H (pages 953-955) and J (pages 959-961) of Volume 4 of the study report

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Table 8. F1a and F1b Pup Body-Weight Gain (grams)✖				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a male pups Lactation days				
1-4[a]	2.2	2.3	2.3	1.5 [68]♪
4[b]-7	3.9	3.9	3.9	2.1 [54]
7-14	10.6	10.3	9.5	8.7 [82]
14-21	10.1	9.6	9.6	9.5 [94]
21-28	19.2	17.1 [89]	17.1 [89]	12.4 [65]
4-28	43.8	40.9 [93]	40.1 [92]	32.7 [75]
F1a female pups Lactation days				
1-4	2.3	2.3	2.3	1.6 [70]
4-7	3.7	3.8	3.8	2.2 [59]
7-14	10.3	10.0	9.2	8.5 [83]
14-21	9.4	9.0	9.3	9.5
21-28	17.7	15.5	16.0	12.8 [72]
4-28	41.1	38.3 [93]	38.3 [93]	33.0 [80]
F1b male pups Lactation days				
1-4	2.7	2.8	2.5 [93]	0.7 [26]
4-7	3.9	4.1	3.9	2.0 [51]
7-14	11.5	11.4	10.9 [95]	8.7 [76]
14-21	10.7	10.4	9.9 [93]	10.4
21-28	21.4	16.3 [76]	14.6 [68]	14.8 [69]
4-28	47.5	42.2 [89]	39.3 [83]	35.9 [76]
F1b female pups Lactation				
1-4	2.8	2.7	2.4 [86]	1.2 [43]
4-7	3.6	3.9	3.6	1.7 [47]
7-14	10.8	11.0	10.5	7.9 [73]
14-21	9.8	9.7	9.2	9.9
21-28	18.7	14.8 [79]	13.3 [71]	14 [75]
4-28	42.9	39.4 [92]	36.6 [85]	33.5 [78]

4[a] day 4 pre-cull; 4[b] day 4 post-cull; ♪ [% of control]; ✖ calculated by this reviewer using data from Tables 7-8, pages 20-21 of original DER [no statistics performed]

Table 9. F1a and F1b Pup Body Weights [grams]✖				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a male pups Lactation days				
1	5.5±0.8 (21)	5.6±0.7 (25)	5.6±0.6 (23)	4.9±0.5 (19)[89]♪
4	7.7±1.5	7.9±1.2	7.9±0.7	6.4±0.7
4	8.0±1.1	7.9±1.1	7.9±0.7	6.4±0.7
7	11.9±1.1	11.8±1.8	11.8±0.8	8.5±1.3
14	22.5±1.7	22.1±2.7	21.3±2.3	17.2±2.1
21	32.6±2.8	31.7±3.1	30.9±2.8	26.7±2.2
28	51.8±6.3 (20)	48.8±6.4 (25)	48.0±5.6 (23)[93]	39.1±3.2 (19)[75]
F1a female pups Lactation days				
1	5.2±0.7 (21)	5.4±0.7 (25)	5.4±0.8 (24)	4.7±0.4 (20)[90]
4	7.5±1.5	7.7±1.2	7.7±0.6	6.3±0.9
4	7.7±0.9	7.7±1.3	7.7±0.6	6.3±0.9
7	11.4±1.0	11.5±1.8	11.5±0.6	8.5±1.5
14	21.7±1.9	21.5±2.6	20.7±2.3	17.0±2.6
21	31.1±2.9	30.5±2.7	30.0±2.8	26.5±3.1
28	48.8±5.3 (20)	46.0±5.5 (25)	46.0±5.3 (24)[94]	39.3±6.3 (20)[81]
F1b male pups Lactation days				
1	5.8±0.4 (23)	5.6±0.6 (25)	5.4±0.5 (23)	4.5±0.4 (8)[78]
4	8.5±0.8	8.4±0.9	7.9±0.6	5.2±1.1
4	8.5±0.8	8.4±0.9	7.9±0.6	5.2±1.2
7	12.4±1.2	12.5±1.3	11.8±0.9	7.2±1.8
14	23.9±2.3	23.9±2.2	22.7±1.2	15.9±3.6
21	34.6±3.6	34.3±3.3	32.6±2.3	26.3±4.2
28	56.0±8.9 (23)	50.6±5.2 (24)	47.2±7.3 (23)	41.1±6.6 (5)[73]
F1b female pups Lactation day				
1	5.3±0.5 (23)	5.3±0.6 (24)	5.2±0.5 (23)	4.4±0.5 (8)[85]
4	8.1±0.8	8.0±0.8	7.6±0.6	5.6±0.8
4	8.1±0.8	8.0±0.8	7.6±0.6	5.5±0.8
7	11.7±1.1	11.9±1.2	11.2±0.7	7.2±1.2
14	22.5±1.9	22.9±1.8	21.7±1.0	15.1±1.0
21	32.3±3.0	32.6±2.6	30.9±2.1	25.0±0.9
28	51.0±7.5 (23)	47.4±4.6 (24)	44.2±6.8 (23)	39.0±1.5 (5)[76]

(# litters); ♪ [% of control]; ✖ data from appendices Y and Z, pages 294-301 of the report

Table 10. F2a and F2b Pup Body Weights [grams]				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F2a male pups Lactation days				
1	5.9±0.6	5.8±0.4	5.7±0.3	-
4	8.7±0.9	8.6±1.0	8.5±0.6	-
4	8.7±0.9	8.6±1.0	8.5±0.6	-
7	12.6±1.0	12.3±1.2	12.4±1.0	-
14	22.7±2.0	22.5±1.9	23.3±1.7	-
21	32.0±3.0	32.0±3.7	32.6±2.9	-
28	55.9±7.6	57.9±6.3	58.2±5.1	-
F2a female pups Lactation days				
1	5.7±0.6	5.4±0.5	5.4±0.3	
4	8.3±0.9	8.1±1.1	8.1±0.6	-
4	8.3±0.9	8.1±1.2	8.1±0.6	-
7	12.1±1.1	11.7±1.3	11.8±0.9	-
14	21.9±1.9	21.7±2.1	22.0±1.6	-
21	30.8±3.1	30.7±3.8	30.7±3.0	-
28	52.5±7.1	53.5±5.8	53.2±4.8	-
F2b male pups Lactation days				
1	5.8±0.4	5.8±0.5	5.8±0.6	
4	8.7±0.8	8.4±1.0	8.6±1.2	-
4	8.8±0.8	8.4±1.0	8.7±1.2	-
7	12.4±1.0	12.5±1.2	12.8±1.9	-
14	22.5±1.4	22.3±1.7	23.2±2.5	-
21	33.2±2.4	33.3±1.9	34.4±4.1	-
28	55.1±5.6	53.2±4.2	56.1±7.9	-
F2b female pups Lactation day				
1	5.5±0.4	5.6±0.4	5.5±0.5	
4	8.5±1.0	8.3±0.5	8.3±1.2	-
4	8.6±0.9	8.3±0.5	8.2±1.1	-
7	12.1±1.5	12.0±0.9	12.1±1.7	-
14	22.0±2.2	21.6±1.1	22.0±2.5	-
21	32.0±2.9	31.8±1.3	32.1±3.4	-
28	51.8±5.7	51.3±6.5	51.2±6.4	-

♪ [% of control]; data from Tables 35-36, pages 838-841 of the report

Table 11. F2a and F2b Pup Body-Weight Gain [grams]✖				
Generation/Sex/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F2a male pups Lactation days				
1-4	2.8	2.8	2.8	-
4-7	3.9	3.7	3.9	-
7-14	10.1	10.2	10.9	-
14-21	9.3	9.5	9.3	-
21-28	23.9	25.9	25.6	-
4-28	47.2	49.3	49.7	-
F2a female pups Lactation days				
1-4	2.6	2.7	2.7	-
4-7	3.8	3.6	3.7	-
7-14	9.8	10.0	10.2	-
14-21	8.9	9.0	8.7	-
21-28	21.7	22.8	22.5	-
4-28	44.2	45.4	45.1	-
F2b male pups Lactation days				
1-4	2.9	2.6	2.8	-
4-7	3.6	4.1	4.1	-
7-14	10.1	9.8	10.4	-
14-21	10.7	11.0	11.2	-
21-28	21.9	20.0	21.7	-
4-28	46.3	44.9	47.4	-
F2b female pups Lactation				
1-4	3.0	2.7	2.8	-
4-7	3.5	3.7	3.9	-
7-14	9.9	9.6	9.9	-
14-21	10.0	10.2	10.1	-
21-28	19.8	19.5	19.1	-
4-28	43.2	43.0	43.0	-

✖ calculated by this reviewer using data from Tables 35-36, pages 838-841 of the report [no statistics performed]

Table 12. Litter Data [# litters with dead pups and # dead pups on lactation day 0]				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a litters # litters # pups	2 [21]✔ 2 {1/sex}	5 [26] 13 {3 ♂/10♀}	0 [24] 0	5 [21] 6 {3/sex}
F1b litters # litters # pups	5 [23] 5 {3 ♂/2♀}	3 [26] 4 {3 ♂/1♀}	3 [23] 3 {2 ♂/1♀}	15 [20] 59 {23 ♂/36♀}
F2a litters # litters # pups	1 [20] 5 {1 ♂/4♀}	1 [24] 1 {1♀}	2 [23] 2 {1/sex}	- -
F2b litters # litters # pups	3 [18] 3 {2 ♂/1♀}	3 [20] 3 {1 ♂/2♀}	2 [19] 2 {1/sex}	- -

✔ total # litters; data from Appendices W & X [pages 286-293] and [pages 1012-1017] of the report;

Table 13 . Gestation Length				
Generation/Dose/Days	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a mating	22.0±0.4 [2]✔	22.2±0.7 [5 (24)♪]	21.9±0.4 [1]	22.1±0.5 [4]
F1b mating	21.9±0.3 [0]	22.0±0.4 [1]	21.7±0.4 [0]	22.5±0.5** [10]
F2a mating	22.3±0.4 [5]	22.0±0.5 [3]	22.0±0.2 [1]	-
F2b mating	22.0±0.3 [1]	22.1±0.5 [0 (24)]	21.9±0.5 [0]	-

✔ [# 23 days] (♪ 24 days for one low dose dam); Data from Tables 32 [pages 116 and 833] and 33 [pages 117 and 834]; Appendices U & V [pages 278-285] and [pages 1006-1011] of Volumes 1 and 4, respectively, of the report; ** $p<0.01$

4. Reproductive function: There was no assessment of reproductive function.

a. Estrous cycle length and periodicity: Results from the evaluation of vaginal smears were not provided. The study [1985] was performed according to the guidelines proposed in 1978, which did not include these parameters.

b. Sperm measures: Results from the evaluation of sperm parameters were not provided. The study [1985] was performed according to the guidelines proposed in 1978, which did not include these parameters.

5. Reproductive performance: There was no apparent adverse effect on reproductive performance. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the F1a, F1b, F2a, and F2b pups is shown in Table 13.

Table 14. Number of Second Matings				
Generation/Dose	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
F1a mating	0	5	1	0
F1b mating	6	6	2	2
F2a mating	3	2	4	-
F2b mating	4	1	4	-

Data from page 14 of the original DER [Document No. 005446]

Reproductive Performance

Observation/Dose Group	Table 15. F0 Generation - Litter F1a			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	5.05±2.46√ [4.0]✖	5.8±2.9 [5.7]	4.8±2.1 [4.8]	5.2±2.8 [4.8]
MALES				
Number mated	30	30	30	30
Number fertile	21 [70%]	25 [83.3]	24 [80]	21 [70]
Fertility not determined	na	na	na	na
Intercurrent deaths	na	na	na	na
FEMALES				
Number mated	30	30	30	30
Number fertile	21 [70]	26 [86.7]	24 [80]	21 [70]
Fertility not determined	na	na	na	na
Intercurrent deaths	1 {day 3}	0	0	0
Mean (±SD) gestation interval (days)	22.0±0.4	22.2±0.7	21.9±0.4	22.1±0.5
Number of litters	21	24	24	21

√ mean calculated by reviewer using data from Appendix S (pages 270-273); ✖mean from Table 30 page 114 in the study report]. Data also from Tables 28 and 32, pages 112 and116; na not available

Observation/Dose Group	Table 16. F0 Generation - Litter F1b			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	5.0±2.2√ [4.6]✖	5.36±3.67 [5.2]	4.5±2.1 [4.8]	4.1±2.4 [4.3]
MALES				
Number mated	29	30	30	30
Number fertile	23	25	23	21
Fertility not determined	na	na	na	na
Intercurrent deaths	na	na	na	na
FEMALES				
Number mated	29	30	30	30
Number fertile	23	27	23	21
Fertility not determined	na	na	na	na
Intercurrent deaths	0	1 {day 23}	0	1 {day 20}
Mean (±SD) gestation interval (days)	21.9±0.3	22.0±0.4	21.7±0.4	22.5±0.5**
Number of litters	23	25	23	10

√ mean calculated by reviewer using data from Appendix T [pages 274-277]; ✖mean from Table 30 page 114 in the study report] Data from Tables 29, 31 and 33, pages 113, 115 and 117 of study report. * p<0.01.

Observation/Dose Group	Table 17. F1 Generation - Litter F2a			
	Control	LDT	MDT	HDT
Mean (±SD) precoital interval (days)	4.8±2.3√ [4.6]✖	4.0±2.3 [4.1]	5.0±3.0 [4.3]	-
MALES				
Number mated	30	30	30	-
Number fertile	21	24	22	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
FEMALES				
Number mated	29	30	30	-
Number fertile	21	24	23	-
Fertility not determined	na	na	na	-
Intercurrent deaths				
Mean (±SD) gestation interval (days)	22.3±0.4	22.0±0.5	22.0±0.2	-
Number of litters	20	24	22	-

✖ Data from Table 29 [page 830] of study report. √Calculated by reviewer using data in Appendix S, pages 994-999

Observation/Dose Group	Table 18. F1 Generation - Litter F2b			
	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day
Mean (±SD) precoital interval (days)	3.3±2.2 √ [3.2]✖	3.1±1.7 √ [3.4]	4.1±3.3 √ [4.0]	-
MALES				
Number mated	30	30	30	0
Number fertile	18	20	20	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
FEMALES				
Number mated	28	30	30	0
Number fertile	18	20	20♪	-
Fertility not determined	na	na	na	-
Intercurrent deaths	na	na	na	-
Mean (±SD) gestation interval (days)	22.0±0.3	22.1±0.5	21.9±0.2	-
Number of litters	18	20	20♪	-

√ mean calculated by reviewer using data from Appendix T (pages 1000-1005); ✖mean from Table 30 [page 831] in the study report]. Pre-coital interval ≥5 days [control 28%; low 40%; mid 55%]; ♪{data are listed for 20 in Appendix T (pages 1004-1005) and Table 30 (page 831); for 19 on pages 833 and 1011

Observation/Dose Group	Table 19. Litter parameters for F_1 generation			
	0 mg/kg/day	5 mg/kg/day	2 0 mg/kg/day	80 mg/kg/day
F_{1a} Generation				
Mean implantation sites	na	na	na	na
Number born live	214	258 ✗✗	240 ♬	183
Number born dead	3	20	3	9
Sex Ratio Day 0 (% ♂)	47	51	50	57
# Deaths Days 0-4 (%)	5	11	3	8
# Deaths Days 4-21 (%)	4	1	0	4
Mean litter size Day 0	10.2	9.9	10.0	9.2 [90]✔
Day 4 [b]	9.9	9.9	9.9	8.8 [89]
Day 4 [c]	8.0	7.6	7.6	7.4 [93]
Day 7	7.8	7.6	7.6	7.3 [94]
Day 14	7.8	7.6	7.6	7.2 [92]
Day 21	7.8	7.6	7.6	7.2 [92]
Live birth index	98.6	92.6**	98.8	95.2
Viability index	98.6	95.2	100	96.8
Lactation index	97.5	99.5	100	97.3
F_{1b} Generation				
Mean implantation sites	na	na	na	na
Number born live	224	266	241	161
Number born dead/dying by day 1	5	4/11	3	59/51
Sex Ratio Day 0 (% ♂)	51	48	46	47
# Deaths Days 0-4 (%)	5	20	4	117✦
# Deaths Days 4-21 (%)	0	1	0	12 (68%)
Mean litter size Day 0	9.5	10.0	10.4	5.1**
Day 4 [b]	9.5	9.8	10.3	4.4
Day 4 [c]	7.1	7.4	7.6	5.3
Day 7	7.1	7.4	7.6	5.7
Day 14	7.1	7.4	7.6	6.0
Day 21	7.1	7.3	7.6	6.0
Live birth index	97.8	94.4	98.8	31.7**
Viability index	100	98.0	99.6	86.3**
Lactation index	100	99.4	100	71.4**

a Data obtained from Appendices W & X, pages 286-293 and Tables 34-35 [pages 118-121]of study report.
✗ listed as 25 in Table 32 (page 118); ✗✗ listed as 238 in Appendix W, page 287 (day 0) and 251in Table 34 on page 118 [live litter size]; ♬ listed as 226 in Appendix W, page 288 (day 0) and 237 in Table 34 [live litter size];
✔ total # litters; data from Appendices W & X [pages 286-293] and [pages 1012-1017] of the report;
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
✦ 12/20 total litter loss

Observation/Dose Group	Table 20. Litter parameters for the F_2 generation			
	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	-
F_{2a} Generation				
Mean implantation sites	na	na	na	-
Number born live	187	220	207	-
Number born dead	5	3	3	-
Sex Ratio Day 0 (% ♂)	51.9	48.2	53.6	-
# Deaths Days 0-4 (%)	2 (1)	2 (0.9)	0	-
# Deaths Days 4-21 (%)	0	1 (0.6)	0	-
Mean litter size Day 0	9.8	9.2	9.0	-
Day 4 [b]	9.7	9.1	9.0	-
Day 4 [c]	7.4	7.0	7.3	-
Day 7	7.4	7.0	7.3	-
Day 14	7.4	7.0	7.3	-
Day 21	7.4	7.0	7.3	-
Day 28	7.4	7.0	7.3	-
Live birth index	97.4	98.7	98.6	-
Viability index	98.9	99.1	100	-
Lactation index	100	99.4	100	-
F_{2b} Generation				
Mean implantation sites	na	na	na	-
Number born live✖	156	186	165	-
Number born dead	3	3	2	-
Sex Ratio Day 0 (% ♂)	46	47	49	-
# Deaths Days 0-4 (%)	4	1	0	-
# Deaths Days 4-21 (%)	0	3	0	-
Mean litter size Day 0	8.6	9.3	8.7	-
Day 4 [b]	8.9	9.3	8.7	-
Day 4 [c]	7.3	7.3	7.2	-
Day 7	7.3	7.2	7.2	-
Day 14	7.3	7.2	7.2	-
Day 21	7.3	7.2	7.2	-
Live birth index	97.5	98.4	98.8	-
Viability index	97.4	99.5	100	-
Lactation index	100	98.6	100	-

Data obtained from Appendices W & X, pages 1012-1017 and Tables 34 and 36 [pages 836-7, 840-841] of study report.
✔ total # litters;
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
✖ the number of pups on day 0 was less than the number on day 1

3. <u>Sexual maturation</u> (F_1): Sexual maturation was not assessed.

<u>Discrepancies</u>: The data presentation is confusing. For example, there are several instances where the total number of pups increases between day of birth [day 0] and day 1. In Appendix W [page 286], the number dead on day 0 in the control group [F1a litters] is listed as 2 [1 male, 1 female]; the number surviving [born live] on day 0 is listed as 214 out of 216. For survival on day 1, it is not clear why the total number of pups is listed as 213 out of 216 instead of 213 out of 214 [survival on day one should be of those that were alive on day 0]. For the 5 mg/kg/day

dose group, the number of pups on day 0 for two litters is less than the number listed on day 1 [dam #5506 had 1 pup on day 0 and 12 listed for day 1; dam # 5534 had 4 pups on day 0 and 13 on day 1]. Survival on day 0 is listed as 238 out of 251. The denominator for the total alive on day 1 is 271, which is the number apparently born; survival on day 1 is listed as 251 out of 271. However, if the extra 20 pups not listed on day 0 are added to the total live on day 0 [238], the total is 258, which suggests there were 7 deaths between day 0 and 1. For dam #5531, there were 7 out of 8 pups alive on day 0 and 7 out of 8 alive on day 1, but the % survival on day 1 is listed as 88% [should read 100%].

Additionally, there is confusing information on the number of dead pups born. For example, Appendix X [page 293] shows the number of dead pups on lactation day zero to be 23 males and 36 females [59] in the high-dose F1b group, which is different from the number [110] listed in Table 35 [page 120] as the number of dead pups [time frame not stated]. The number of pups dying between day 0 and day 1 was not found in the report, but a comparison of the number of pups listed on day 0 to the number listed for day 1 in the individual data in Appendix X [page 293] gives the additional 51 dead pups listed in Table 35.

Pup Deaths			
# dead on lactation day 0	Appendix W or X	Table 34 or 35	apparent # dying before day 1
F1a control	2	3	1
F1a 5 mg/kg/day	13	20	7
F1a 20 mg/kg/day	0	3	3
F1a 80 mg/kg/day	6	9	3
F1b control	5	5	0
F1b 5 mg/kg/day	4	15	11
F1b 20 mg/kg/day	3	3	0
F1b 80 mg/kg/day	59	110	51

In Appendix S [pages 994-999], the individual F1 data presented are for the F2a mating; however, page 998 has **F2b** mating listed.

CASWELL FILE

3,15

005446

SEP - 8 1986

Subject: The Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats.

From: David G Anderson, PhD. 9/5/86
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

To: Ms. Lois Rossi PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD. ABK 9/8/86
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) 9/8/86

The 2-generation rat reproduction feeding study on 2,4-D has been reviewed and classified as Core-minimum data. The calulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 15(20) mg/kg/day.*
LEL - 58(80) mg/kg/day, reduced male body weight.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered).

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Reviewed by: David G Anderson David G Anderson 9/5/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Effects of 2,4-D on Two-Generations of Reproduction in Rats

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 259442-6 (Study in 5 Volumes)

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid.

AUTHORS: Stanley Kopp, Patricia L Leist, Michael D Mercieca, Elaine J Tasker, Gabriela P Adam, Mark D Nemec, Dean E Rodwell.

STUDY NO.: WIL-81137

TESTING PERIOD: November 16, 1982 to May 15, 1984

REPORT ISSUED: July 26, 1985

PURITY OF TEST SUBSTANCE: ITF analysis 97.5%
WIL analysis 95.8%

CORE GRADE: Minimum.

A. CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels and no effect levels are expressed as the lowest dose level consumed within a measured dose level range. The target or nominal dose levels administered, for reference purposes only, are enclosed in parentheses(Discussed more fully in the section on Study Design and Conduct). Dose levels are given in mg/kg/day.

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LEL and NOEL is expressed in mg/kg/day

F0 parental toxicity
LEL- 58(80), reduced body weight.
NOEL- 15(20)
F1 parental toxicity
LEL- 14(20), reduced body weight.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

In designating the LEL and the NOEL, several considerations were applied. The lowest dose level in a range was used. Although it might be expected that the highest dose level within a range would initiate the toxicity, in a study on reproduction, where effects may be development stage or age specific as well as dose dependent, the highest dose level is not totally appropriate. In the study under consideration, the dose levels consumed varied widely during the study, and it was not always possible to determine adequately the dose level or the animal state at which the toxicity was initiated. Thus, it seems appropriate to select, for the LEL, the lowest dose level possibly resulting in the effect.

The NOEL is also designated as the lowest dose level in the range where no effects were observed. The upper dose level of the range was rejected because the animals did not continuously consume these levels. If they had, effects may have been demonstrated. Thus, for safety considerations, the lowest dose level within the range where no effects were observed is designated the NOEL.

The appropriate dose level range for the NOEL for the F1 female body weight reduction includes; a) the gestation and lactation for the F1b pups(the F1 females were selected from these pups), b) and the growth and develment of the F1 females, c) and the gestation and lactation for the F2a and F2b litters, e) and for the 4 weeks of dosing after weaning the F2b litters. The lowest dose level consumed during these periods is considered to be the NOEL.

Similarly, the NOEL for the F1b pups is the lowest dose level consumed by F0 dams during the gestation and lactation for the F1b litters.

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The effect and no effect levels from this study are also presented as the target dose levels and the range in the amount of test substance consumed. The target dose levels are the dose levels which were designed for the study and which the testing laboratory attempted to deliver to the animals. The amounts of test substance consumed are the actual dose-levels delivered to the animals, at least as best could be determined from the concentration of the test substance in the feed, food consumptions, and the animal weight for the week concerned. Since the dose levels are calculated for 1 week before they are delivered, the actual delivered dose varied somewhat from these anticipated dose-levels during the study.

Effect and No Effect Levels, with ranges

F0 parental LEL and NOEL in mg/kg/day
- LEL- 80(58-94)([a])([b]), F0 male body weight reduction.
- 80(71-86)([b]) , F0 female body weight reduction.
- 80(69-114)([c]) , F0 increase in length of gestation.
- NONE , F0 and F1 fertility.
- NOEL- 20(15-22)([b]) , No F0 male body weight reduction occurred.
- 20(18-21)([b]) , No F0 female body weight reduction,
- 20(18-35)([c]) or no increased length of gestation occurred.

F1 parental LEL and NOEL in mg/kg/day
- LEL- 20(14-48)([d]) , F1 female body weight reduction.
- NOEL- 5(3.8-13.5)([d]), No F1 female body weight reduction occurred.

Developmental toxicity in mg/kg/day to dams
- LEL- 80(69-112, gestation and lactation for the F1a litters)([e]), F1a pup death.
- 80(103-133, gestation and lactation for the F1b litters)([e]), F1b pup death.
- 80(69-112, gestation and lactation for the F1a litters)([e]), F1a reduced pup weight.
- 20(26-48, gestation and lactation for the F1b litters)([e]), F1b reduced pup weight.
- 80(103-114, gestation producing the F1b litters)([e]), F1b skeletal anomalies, and reduced ossification, the only dose level studied.
- NOEL- 5(7.2-13.5, gestation and lactation for the F1b litters)([e]), for all developmental effects.

Discounted effects and toxicity

F0 male liver and liver/body weight ratio reduction at all dose levels.

F0 female kidney and kidney/body weight ratio increase at all dose levels.

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The following dose levels were administered.

F0 males[f]	F0 females[f]
5(3.7-6.1)	5(4.2-8.6)
20(14.9-24.5)	20(17.8-29.5)
80(57.7-103.7)	80(70.7-124.5)
F1 males[f]	**F1 females[f]**
5(4.7-5.6)	5(4.5-6.0)
20(18-23)	20(19-24)
80[g]	

([a]) Target dose level(range in amount of test substance consumed, except during gestation and/or lactation, unless noted)
([b]) Includes test substance consumption prior to mating only.
([c]) Includes test substance consumption prior to mating, through gestation and lactation for the F1a litters and the gestation producing the F1b litters, the only gestation for which the effect was noted.
([d]) Includes test substance consumption throughout life time of the the F1 generation, which includes the F1b, F2a, and F2b litters.
([e]) Includes test substance consumption only during the period/s indicated.
([f]) Target dose levels administrated(range in test substance consumption for the test animals indicated, except for gestation and lacation) in mg/kg/day.
([g]) Due to excess toxicity the F1b litters, the highest dose level was not continued beyond weaning.

The dose levels were set at 50% of the premating dose during the second week of lactation and 33% of the premating dose during the third and forth week of lactation. This somewhat arbitrary setting of dose-levels during midlactation and end lactation, has merit but needs evaluation for its impact on Agency assessment of reproductive effects. Also, the consequence of the reduced dosing to young animals when the study was initiated and just after weaning needs evaluation. Animals eat approxmately twice as much food as they do as adults during the first 2-3 weeks post-weaning. Thus, they consumed less test substance in this study than would have if the concentration of the test substance in the feed had not been adjusted for body weight.

B. Conclusions

Toxicity was expressed in the F1b pups and in F1 females at dose levels lower than those administered to the F0 parents. Pup death occurred at birth and before lactation day 4 in F1a and F1b litters at the highest dose level which caused slight but statistically significant reduced weight gain in the F0 parents. Because of the toxicity to the pups at this target dose level of 80 mg/kg/day, this dose level was dropped from the study after weaning the remaining F1b pups. Reduced weight gain occurred in F1b pups during lactation at the middle dose level. At this same dose level, the F1b female pups, which became the F1 female generation, demonstrated a reduced body weight compared with controls during the last 4 weeks before sacrifice, but after weaning the F2b pups No significant effects occurred in any pups or any animals at the lowest target dose level. No reduced food consumption occurred to explain any of these effects on weights.

At all dose levels, absolute and relative liver weights were statistically significantly less than controls in F0 males and absolute and relative kidney weights at all dose levels were statistically significantly greater than controls in F0 females. These statistically significant effects did not demonstrate "smooth" dose response curves, and the effects were not confirmed in the F1 generation or in the histological examination of these organs. The report did not consider them to be biologically significant.

The toxicological significance of these effects are discounted. The liver weight reduction was not seen in 90 day subchronic and chronic studies conducted in this species and strain of rats. The increased kidney weights are also discounted because the kidney weights of 5 female controls were lower than the kidney weights of the remaining control animals of the F0 generation by approximately 3 standard deviations. If these animals are excluded from the average, then the kidney weights of dosed animals are comparable to the kidney weights of the remaining animals in the control group.

The LEL for development is reduced pup weight compared to controls during gestation and lactation of F0 dams at a target dose level of 20 mg/kg/day or a dose level range of

26-48 mg/kg/day. At the highest dose level, pup viability was reduced in the F1a and F1b litters. The NOEL for the reduced pup weight in the F1b litters compared to controls is a dose range of 7.2-13.5 mg/kg/day during gestation and lactation of F0 females.

Since the liver weight decrease in males,and the kidney weight increase in females is not considered biologically significant, the LEL in adults is in F1 females at the target dose level of 20 mg/kg/day, where statistically significant weight depression compared to controls occurred during the last 4 weeks before sacrifice,but after weaning the F2b pups. The NOEL for F1 adults then would be the target dose level of 5 mg/kg/day, the same target dose level as the NOEL for the F1b pup weight reduction. However, the range of dose levels consumed differed(see LEL and NOEL above).

No effects were seen on fertility in the F0 or the F1 males or females.

C. Study Design and Conduct

The study was conducted essentially according to the OPP guidelines proposed August 22, 1978, for a two-generation, two litters per generation study of reproduction. The quality assurance statment was signed the director of quality assurance, Ralfh Anderson, on 7/26/85.

About 140 Fischer 344 rats per sex were obtained from Charles River Breeding Laboratories, Inc, Kinston, NY on November 3, 1982, and quarantined for 13 days. Assignment of 30 rats per group were based on random selection of rats in a block design for body weight stratistication. Animals were housed individually under recommended conditions.

The F0 generation was placed on diets designed to deliver dose levels of 0, 5, 20, or 80 mg/kg/day, respectively, to each group, each of 30 rats per sex, for 105 days prior to mating. Subsequently, the animals were dosed in an analogous manner during each mating, each gestation, and each lactation. The total dosing and continuous dosing period for F0 animals was 40 weeks which included 2 weeks rest between the end of lactation for the F1a litters to the beginning of mating for the F1b litters and 30 days after weaning these latter litters.

The F1 generation, selected from the F1b pups, was exposed to the test substance in utero, and continuously via the

milk or the feed for 125 days postnatally and prior to mating and through mating, gestation and lactation for the F2a litters. Dosing continued through a 2 week rest period and mating, gestation, lactation for the F2b litters and for at least 30 days after weaning the F2b litters.

The total period of continuous administration of the test substance, from initial dosing of the F0 generation to the end of the F1 generation, was 77 weeks. During this period, the test substance was administered to the F0 generation, F1a and F1b litters, the F1 generation(selected from F1b litters), including the F2a and F2b litters and for 30 days after weaning the F2b litters.

The test substance was administered in the feed at target dose levels of 5, 20, or 80 mg/kg/day. The concentration in the feed was adjusted weekly according to the food consumption during the previous week and the average body weight for that week. This regimen was followed in the F0 generation up to week 15 (105 days) or just prior to mating to produce the F1a litters. Except as indicated below, monthly adjustments were made after mating. During mating, males and females were exposed to the diet prepared for the females which was based on the concentration prepared for the week prior to mating (week 15 for the F0 matings). The same dietary concentration was used throughout mating, gestation, and the first week of lactation. During the second week of lactation, the dietary concentration was reduced by 50 percent and during the third and fourth weeks of lactation, the dietary concentration was reduced by 67 percent of diet concentrations used during the first week of gestation (a concentration based on week 15). A similar dosing regimen was followed in producing the F1b litters, except the dosing regimen was based on body weights for week 24 and food consumption for week 15. The actual dose level consumed during gestation and lactation are given in tables 1 and 2.

The report claimed that the food consumption for week 15 was actually for 6 days instead of 7, but that the average daily food consumption used for week 24 was incorrectly based on a 7 day week. Thus, the average daily food consumption for week 15 was calculated to be 86%(6/7=.86) of the actual daily average. This would result in the intended concentration of test substance in the feed during production of the F1b litters to be 86% of the actual feed concentration used during this period. The report did not make it clear whether or not this same error was made in the test substance concentration in the feed used during production of the F1a litters.

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Table 1.

Test substance consumed during gestation in F0 and F1 dams producing F1a, F1b, F2a, and F2b litters.

		Target dose levels in mg/kg/day			
		0	5	20	80
		Test substance consumed, in mg/kg/day, was calculated from the concentration, food consumption, and body weight.			
F0 dams during the gestation producing the F1a	days 0-7	--	4.6	18.1	69.0
	days 7-13	--	5.0	20.5	79.6
	days 13-20	--	4.9	19.6	76.1
F0 dams during the gestation producing the F1b	days 0-7	--	7.2	26.4	103.4
	days 7-13	--	8.0	29.4	113.8
	days 13-20	--	7.5	28.4	106.9
F1 dams during the gestation producing the F2a	days 0-7	--	3.8	17.1	NC
	days 7-13	--	4.8	19.6	NC
	days 13-20	--	5.1	19.9	NC
F1 dams during the gestation producing the F2b	days 0-7	--	3.9	14.2	NC
	days 7-13	--	4.8	18.1	NC
	days 13-20	--	4.7	16.7	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

Table 2

Test substance consumed during lactation in F0 and F1 dams for F1a, F1b, F2a, and F2b litters.

		Target dose levels in mg/kg/day				
			0	5	20	80
		Test substance consumption in mg/kg/day calculated from concentration, food consumption, and body weight. ([a])				
F0 dams during lactation for F1a	days	1-7	--	8.9	34.7	112.3
	days	7-14	--	6.5	25.2	84.0
	days	13-20	--	4.9	19.6	76.1
	days	14-21	--	5.1	20.7	72.4
	days	21-28	--	6.9	27.6	82.9
F0 dams during lactation for F1b	days	1-7	--	13.5	47.8	132.7
	days	7-14	--	9.4	34.6	106.9
	days	14-21	--	7.3	26.4	90.9
	days	21-28	--	9.3	32.6	112.7
F1 dams during lactation for F2a	days	1-7	--	8.2	34.0	NC
	days	7-14	--	5.9	24.6	NC
	days	14-21	--	4.6	18.8	NC
	days	21-28	--	7.0	29.1	NC
F1 dams during lactation for F2b	days	1-7	--	7.6	28.5	NC
	days	7-14	--	5.6	20.6	NC
	days	14-21	--	4.8	17.5	NC
	days	21-28	--	6.6	25.2	NC

NC-Testing of the F1 generation was not continued at this dose level after weaning.

([a]) Included in these values is the 50% reduction in the concentration of the test substance during the second week and the 67% reduction during the third and fourth week of lactation.

The F1b litters for the F1 generation remained on the reduced diet of lactation week 3 and 4 after weaning from the F0 dams. After selection for the F1 generation, pups were placed on test diets at target dose levels of 5 and 20 mg/kg/day, with weekly adjustments to week 53, the week prior to mating to produce the F2a litters. At this time, F1 males received the same diet as F1 females. Monthly adjustments were made to the diets after mating, except during the second week of lactation for the F2a and F2b litter when the concentration of the test substance was reduced by 50 percent and during the third and fourth weeks of lactation for the same litters when the concentrations of the test substance was reduced by 67 percent.

The study report did not specifically state how the doses were adjusted for the F1 generation but their dosing regimen can be calculated from the amounts of test substance consumed, and the body weights during the F1 generation for gestation and lactation for the F2a and F2b litters. The study report stated, "After selection, the F1 pups were placed on test diets at dose levels of 5 and 20 mg/kg/day." The study report also stated that adjustments were made to the diet based on food consumption and body weight.

These dosing regimens resulted in generally higher dose levels in the lactating F0 and F1 dams than for the premating target dose levels. The higher dose levels were greatest in F0 dams lactating for the F1b litters. Table 1 and 2 gives the amount of test substance consumed during gestation and lactation, respectively for F1a, F1b, F2a, and F2b litters. Consumption of test substance during gestation is included in table 1 because test substance consumption differed among these litters. The downward adjustment of the concentration in the feed during lactation did not occur during gestation. At other times the actual dose levels consumed were very close to the target dose levels.

The usual parameters were evaluated such as, fertility, duration of gestation, viability of pups at parturition and during lactation, the amount of food consumption, body weight, pup anomalies, and variations, in addition to histopathology on the testes, ovaries, kidneys, and livers. Organ weights were determined on the kidney, liver at necropsy and on testes after fixing in 10 percent formalin.

The following organs and tissues were taken at sacrifice and preserved, but histopathology was conducted only as previously indicated.

1. Adipose tissue
2. Adrenals
3. Aorta
4. Bladder
5. Bone marrow
6. Brain
7. Cecum, colon
8. Spinal cord
9. Epididymis
10. Esophagus
11. Eyes
12. Ovaries/Testes
13. Heart
14. Intestines
15. Kidneys
16. Liver
17. Lung/bronchi
18. Lymph node-thoracic and mesenteric
19. Mammary Gland and Skin
20. Nasal turbinates
21. Pancreas
22. Parathyroids
23. Pituitary
24. Prostate
25. Salivary Glands
26. Sciatic Nerve
27. Seminal Vesicle
28. Skeletal Muscle
29. Spinal Cord
30. Spleen
31. Sterum
32. Stomach
33. Thymus
34. Thyroid
35. Trachea
36. Uterus/cervix
37. Vagina

Statistical Methods

All analyses were conducted using two-tailed tests (unless otherwise specified).

1. Histopathological findings and incidence by sex were compared to control groups by Kalmogorov-Smirnov one-tailed test.

2. F0 and F1 male and female fertility indexes, F1a, F1b, F2a, and F2b pup sex ratios on lactation day 1, and F1a, F1b, F2a, and F2b pup survival indexes on on lactation day 4, 7, 14, 21, and 28 for the control groups were compared to each treated group by the Chi-square test with Yates correction factor.

3. Other effects in treated groups were compared to controls by analysis of variance followed by Dunnett's test.

Summary of Study Conduct

1. Test substance administered continuously throughout all phases of the study.

2. F0 dosed continuously from approx. 5 to 6 weeks of age for 105 days prior to first mating (i.e., approx. 20 weeks of age).

3. F0 mated 1:1 for 10 days and if no evidence of sperm, second matings were allowed with a proven male for 5 days.

4. F0 continued for 3 weeks of gestation and 4 weeks to weaning of F1a litters. Pups reduced to 8 per dam on day 4 of lactation.

5. All F1a litters necropsied and discarded after weaning.

6. F0 rested 2 weeks between weaning F1a and mating for production of the F1b as in #3.

7. F0 continued for 3 weeks gestation and 4 weeks to weaning of F1b litters. Pups reduced to 8 per dam on day 4 of lactation.

8. Ten F1b pups per sex per dose level randomly selected for necropsy, after weaning.

9. One pup per sex per dam per dose level randomly selected from F1b litters for the F1 generation. Because of excess toxicity at the target dose level of 80 mg/kg, only controls, and the target dose level groups of 5 and 20 mg/kg were continued on study. All F1b pups at 80 mg/kg/day were sacrified at the end of weaning.

10. All F0 animals were sacrificed on week 40 of the study.

11. Selected F1 pups were dosed via milk and in the feed for 125 days prior to mating to produce the F2a litters.

12. Dosing, mating, gestation, and weaning in the F1 generation producing the F2a and F2b litters followed procedures, including necropsy, similar to those followed for the F0 generation in producing the F1a and F1b litters.

13. All F1 animals were sacrificed on week 77 of the study.

14. All F1a, F1b, F2a, and F2b dying prior to weaning were studied for malformations and variations.

D. Test Chemical Identity and Concentration in the Feed

The study report, presented an analysis conducted by Wil Research Laboratories, and the Industry Task Force analysis on 2,4-D. According to a Wil Research analysis, the test substance was 95.8 percent pure 2,4-D. The report presented the following analysis of the test substance by the task force, but no further analysis or explanation of the differences between the Task Force analysis of 97.5 percent, and the Wil Research analysis of 95.8 percent, was presented.

2,4-D 97.5%

[redacted]

ND = Not detected, (lowest level detectable).

Samples of the diets containing 2,4-D were collected for study weeks 0, 1, 2, 3, 4, 8, 13, 26, 39, 52, 65, 77. None of the sample diets were collected during weeks of gestation or lactation. The analyses after recovery of 2,4-D from the diets with the highest concentration were within 10 percent of the measured concentration. Analyses of 2,4-D in the diets at the middle dose level and the lowest dose level were always within 15 percent to 20 percent of the measured concentrations, except for three of the lowest dose levels which were 77 percent, 61 percent, and 55 percent of the measured dose levels. One was in a diet mixed on the 4th week of the study and two were for a diet mixed on the 13th week of the study. The 55 percent of the measured level was apparently a repeat analysis on a sample of the diet yielding the 61 percent of measured dose level.

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E. Results

1. Fertility in F0 and F1 Males and Females.

No reduced fertility was expressed in males or females of the F0 generation in producing either the F1a or the F1b litters. However, a nonstatistically significant apparent reduction in male fertility occurred in producing the F1b litters (table 3). No reduced fertility was expressed in males or females of the F1 generation in producing the F2a and F2b litters. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the F1a/F1b pups were 0/6, 5/6, 1/2 and 0/2 for controls and the target dose levels of 5, 20, or 80 mg/kg/day, respectively. Second matings to produce F2a/F2b pups were 3/4, 2/1, and 4/4 for control and the target dose levels of 5, or 20 mg/kg/day.

The fertility index for production of the F1a and F1b litters is 70 to 79 percent in control F0 males and 70 to 79 percent in control F0 females (see table 3). The fertility index for males and females, respectively is the number of gravid females divided by the number males or females mated, respectively, adjusted to percent. These indexes ranged from 70 to 83 percent in treated males and 70 to 90 percent in treated females producing the F1a and F1b litters. Similarly, the fertility index for production of F2a and F2b litters is 60 to 70 percent in control F1 males and 64 to 72% in control F1 females (table 4.). These indexes range from 67 to 80 percent in treated F1 males and 64 to 80 percent in treated F1 females producing F2a and F2b litters. None were statistically significantly different from controls. The number of days required for mating ranged from 4.0 to 5.7 days of cohabitation to produce the F1a and F1b litters and 3.2 to 4.6 days of cohabitation to produce the F2a and F2b litters. These were no different from control values.

This failure to detect an effect on fertility is consistent with the lack of histopathological findings in the testes or epididymides of males and with the lack of histopathological findings in the ovaries or uteri of females from the F0 or F1 generation at terminal sacrifice. However, since the highest dose level was dropped from the study, fertility in the F1 generation was not evaluated at this dose level. Thus, the mid target dose level of 20 mg/kg/day should be considered the NOEL for fertility.

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Table 3

Fertility indexes for F0 male and females producing F1a and F1b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F1a				Producing F1b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	70	23/29	79	23/29	79
5	25/30	83	26/30	87	25/30	83	27/30	90
20	24/30	80	24/30	80	23/30	77	23/30	77
80	21/30	70	21/30	70	21/30	70	21/30	70

Table 4

Fertility indexes for F1 male and females producing F2a and F2b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F2a				Producing F2b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	72	18/30	60	18/28	64
5	24/30	80	24/30	80	20/30	67	20/30	67
20	22/30	73	23/30	77	20/30	67	20/30	67

2. Length of Gestation in F0 and F1 Females

The lengths of gestation was statistically significantly prolonged in F0 females producing the F1b pups only and only at the highest target dose level of 80 mg/kg/day. This increase in gestational lengths was due to a gestation length of 23 days in approximately one halt of the dams from this group instead of the usual 22 days of gestation demonstrated by most F0 and F1 dams in all groups. The LEL is between 103 and 114 mg/kg/day and NOEL is between 18 and 35 mg/kg/day.

The effect could result from delayed implantation, hormonal imbalance, or parturition problems. The effect is considered biologically significant and undesirable.

3. Body Weights of the F0 and F1 Generations.

The mean body weights of F0 males and female rats were statistically significantly less than controls in the high dose group only. In F0 males, the reduced body weight (97 percent of controls) was consistent after the sixth week of test substance consumption and in F0 females the body weight was consistently reduced (96 percent of controls) by the twelfth week of test substance consumption. The failure to gain as much weight as controls could not be attributed to reduced food consumption. The food consumption, and the food consumption per gram body weight gain was slightly increased. Body weights of the F0 generation in the target dose groups of 5 or 20 mg/kg were similar to control weights throughout this study, but food consumption appeared to be slightly elevated(not satistically significant).

F0 dams producing F1a and F1b litters had statistically significantly lower body weights than control weights on day 20 of the gestation producing the F1a and F1b litters in the highest dose group (table 5). At this dose level, body weights of dams were reduced on day 7, 13, and 20 of the gestation producing F1a litters, but the body weights of dams producing F1b litters were statistically significantly reduced only on day 20. Thus, toxicity was expressed in F0 dams during gestation of the F1a and F1b litters.

On lactation day 7, F0 dams lactating for F1a litters, express significantly reduced body weights in the highest dose group (table 5). For these dams, the body weight per gram of food consumed was about one half the value when compared to other dose groups and controls(data not shown). Dams demonstrated toxicity during lactation for the F1a, and for the F1b litters. At the end of lactation for the F1a and F1b litters, the body weights were satistically significantly elevated.

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Table 5

F0 Female Body Weight (g) during gestation and lactation for F1a and F1b litters.

	Target Dose Levels (mg/kg/day)			
	0	5	20	80
	Body wt. of F0 during gestation producing F1a			
Day 0	178	179	178	173
7	190	191	191	181**
13	208	208	206	196**
20	246	252	249	232*
	Body wt. of F0 during lactation for F1a			
Day 0	189	191	191	184
7	205	207	201	189**
14	212	212	207	208
21	216	213	219	212
28	189	184	185	204**
	Body wt. of F0 during gestation producing F1b			
Day 0	200	205	202	197
7	210	214	210	204
13	226	232	230	218
20	270	277	274	244**
	Body wt. of F0 during lactation for F1b			
Day 0	210	215	208	205
7	226	233	225	211*
14	228	237	233	224
21	229	239	234	231
28	203	197	193	226*

*p < 0.005, Dunnett's Test.
**p < 0.01, Dunnett's Test.

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Table 6

F1 Female Body Weight (g) during gestation and lactation for F2a and F2b litters.

	Target Dose Levels (mg/kg/day)		
	0	5	20
F1 during the gestation producing F2a			
Day 0	201	198	198
7	211	208	211
13	234	227	228
20	271	271	270
F1 during lactation for F2a			
Day 0	216	211	211
7	228	221	222
14	234	232	233
21	232	233	236
28	220	221	223
F1 during gestation producing F2b			
Day 0	222	221	214*
7	234	229	224*
13	250	248	241
20	293	290	278
F1 during lactation for F2b			
Day 0	236	234	227*
7	248	245	237
14	260	245*	248
21	255	252	250
28	228	221	222

*$p < 0.05$, Dunnett's Test.

The body weights of the F1 generation, after selection, was comparable to control body weights, except in females at the target dose level of 20 mg/kg during weeks 74 to 77 where they were statistically significantly less than controls (97 percent of controls). The report stated that these body weight reductions in females were not biologically significant. No explanation was presented.

The body weights of F1 females during gestation and lactation demonstrated no consistently significant patterns during production or lactation for the F2a or F2b litters (table 6), however they were statistically significantly reduced on day 0 and 7 of the gestation producing the F2b litters, and on day 0 of lactation for the the F2b litters.

4. Pup Weights from F1a, F1b, F2a, and F2b Litters

Pup weights were significantly reduced over control weights in the F1a (table 7) and F1b (table 8) pups only. Both male and female F1a and F1b pup weights were less than control weights from birth to lactation day 28 in the 80 mg/kg target dose group. At the next lower dose level, both F1a and F1b male and female pup weights tended to be apparently lower than control weights toward the end of lactation. By day 20 of lactation, both male and female pups in the F1b litters only demonstrated a statistically significant decrease in body weight over control weights. The male pup weight in F1b litters in the lowest dose group which were statistically significantly reduced on lactation day 28 may not be biologically significant, since there were no apparent differences from control weights throughout the previous weeks of lactation.

None of the F2a or F2b pup weights were found to be different from control weights.

Table 7

Summary of Fla litter weights (g)
males and females

Group No.	Dose Level (mg/kg/day)		Lactation Days 1	4 Before Selection	4 After Selection	7	14	21	28
		Males							
1	0	Mean	5.5	7.7	8.0	11.9	22.5	32.6	51.8
		S.D.	0.83	1.50	1.06	1.15	1.72	2.81	6.33
2	5	Mean	5.6	7.9	7.9	11.8	22.1	31.7	48.8
		S.D.	0.71	1.20	1.22	1.83	2.67	3.06	6.37
3	20	Mean	5.6	7.9	7.9	11.8	21.3	30.9	48.0
		S.D.	0.61	0.71	0.71	0.80	2.26	2.59	5.62
4	80	Mean	4.9*	6.4**	6.4**	8.5**	17.2**	26.7**	39.1**
		S.D.	0.46	0.71	7.22	1.30	2.10	2.22	5.24
Group No.		Females							
1	0	Mean	5.2	7.5	7.7	11.4	21.7	31.1	48.8
		S.D.	0.72	1.41	0.94	1.03	1.91	2.87	5.25
2	5	Mean	5.4	7.7	7.7	11.5	21.5	30.5	46.0
		S.D.	0.73	1.24	1.25	1.76	2.55	2.74	5.47
3	20	Mean	5.4	7.7	7.7	11.5	20.7	30.0	46.0
		S.D.	0.75	0.58	0.59	0.66	2.27	2.79	5.28
4	80	Mean	4.7	6.3**	6.3**	8.5**	17.0**	26.5**	39.3**
		S.D.	0.39	0.85	0.85	1.46	2.57	3.10	6.30

* = Significantly different from control group at .05 level using Dunnett's test.

** = Significantly different from control group at .01 level using Dunnett's test.

Table 8

Summary of F1b litter weights (g)
Males and females on
lactation days

Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	1	4 Before Selection	4 After Selection	7	14	21	28
1	0	Mean	5.8	8.5	8.5	12.4	23.9	34.6	56.0
		S.D.	0.43	0.77	0.77	1.17	2.31	3.58	8.87
2	5	Mean	5.6	8.4	8.4	12.5	23.9	34.3	50.6*
		S.D.	0.58	0.92	0.92	1.32	2.19	3.34	5.17
3	20	Mean	5.4	7.9*	7.9*	11.8	22.7	32.6	47.2**
		S.D.	0.50	0.63	0.63	0.86	1.18	2.34	7.26
4	80	Mean	4.5**	5.2**	5.2**	7.2**	15.9**	26.3**	41.1**
		S.D.	0.44	1.14	1.16	1.80	3.57	4.23	6.58
Group No.		Females							
1	0	Mean	5.3	8.1	8.1	11.7	22.5	32.3	51.0
		S.D.	0.46	0.83	0.83	1.11	1.89	2.95	7.52
2	5	Mean	5.3	8.0	8.0	11.9	22.9	32.6	47.4
		S.D.	0.59	0.83	0.84	1.19	1.79	2.59	4.56
3	20	Mean	5.2	7.6	7.6	11.2	21.7	30.9	44.2**
		S.D.	0.46	0.57	0.62	0.68	1.01	2.06	6.77
4	80	Mean	4.4**	5.6**	5.5**	7.2**	15.1**	25.0**	39.0**
		S.D.	0.54	0.83	0.80	1.23	1.00	0.91	1.47

* = Significantly different from control group at .05 level using Dunnett's test.
** = Significantly different from control group at .01 level using Dunnett's test.

The reduced F1b pup body weights in the mid dose level occurred from lactating dams demonstrating no statistically significant toxic signs at the time, although their body weights were apparently reduced from controls on lactation day 28. This may indicate that a change in the metabolism of 2,4-D occurred in F0 dams from production of the F1a to production of the F1b litters. Thus, dams exhibiting apparently no toxicity at the time, resulted in a reduction in pup weight over control weights.

5. Viability of F1a, F1b, F2a, and F2b Litters

The study demonstrated a statistically significantly reduced pup viability over controls only at the highest target dose level of 80 mg/kg (tables 9 and 10). The greatest reduction occurred in F1b pups at birth, with the mean litter size being about one half the control value due to deaths of portions and of entire litters. The mean litter size was reduced from five to three by day 14 of lactation, with no more deaths by lactation day 28 (table 10).

Some indication of reduced litter size was apparent in F1a litters of the target dose of 80 mg/kg, but the apparent decrease was not statistically significant (table 9). At birth however, there was a difference in the sex ratio of pups which was significant at the $p < 0.01$ level. From day 1 to day 28 of lactation, no further significant number of pup deaths occurred.

The study report stated that the decrease in female pups at births in F1a litters was not dose-related. I believe that it may be dose related, since at the highest test substance consumed by mothers producing F1b pups, where test substance consumption was higher than in dams producing the F1a litters, both male and female pup survival at birth were less in these F1b pups than the corresponding pup survival in the F1a pups. Thus, there appeared to be a dose response relationship.

Viability of the F2a and F2b pups was not affected.

6. Malformations and Variations

F1b pups which died before lactation day 28 were studied for malformation and variation. As can be seen from table 11, bent ribs, 14 the rudimentary ribs, malaligned sternebrae and unossitied sternebrae were seen in the F1b pups. Since most of these pups died at birth or were dead by day 1 of lactation, the effects were seen primarily just after birth at the highest dose only and in the F1b pups only. This was the only group for which there were sufficient deaths, and animals could be necropsied. Only pups which died were available for necropsy except at weaning. These effects are

Table 9
Summary of Fla viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	3	99:114	213/21	10.1	213/216	98.6	208/213	97.7	160/160	100.0
2	20	133:118	251/25	10.1	251/271**	92.6	247/251	98.4	191/191	100.0
3	3	121:116	237/24	9.9	237/240	98.8	237/237	100.0	183/183	100.0
4	9	109:71**	180/20	9.0	180/189	95.2	175/180	97.2	147/147	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	156/160	97.5	156/160	97.5	156/160	97.5	156/160	97.5
2	190/191	99.5	190/191	99.5	190/191	99.5	190/191	99.5
3	183/183	100.0	183/183	100.0	183/183	100.0	183/183	100.0
4	146/147	99.3	143/147	97.3	143/147	97.3	143/147	97.3

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival ratios and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different form control at .01 level.
Live litter size = No. pup alive on day 1 of lactation/no. litters.
Gestation index = No. pups alive on day 1 of lactation/total no. pups born.
Viability indexes = No. pups alive on day 4 before selection/no. pups alive day 1.
= No. pups alive day n/no. pups alive day 4 after selection.

Table 10
Summary of F1b viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	5	112:107	219/23	9.5	219/224	97.8	219/219	100.0	164/164	100.0
2	15	120:131	251/25	10.0	251/266	94.4	246/251	98.0	177/177	100.0
3	3	110:128	238/23	10.4	238/241	98.8	237/238	99.6	174/174	100.0
4	110**	23:28	180/20	5.1**	51/161**	31.7	44/51**	86.3	42/42	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	164/164	100.0	164/164	100.0	164/164	100.0	164/164	100.0
2	177/177	100.0	177/177	100.0	176/177	99.4	176/177	99.4
3	174/174	100.0	174/174	100.0	174/174	100.0	174/174	100.0
4	34/42**	81.0	30/42**	71.4	30/42**	71.4	30/42**	71.4

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different from control at .01 level.
Live litter size , gestation index and viability indexes = see legend table 9.

sometimes seen at dose levels causing maternal toxicity, but administration of many compounds do not cause these effects at maternally toxic dose levels.

The number of malformations and variations in these F1b pup dying prior to weaning were apparently not sufficient for statistical significance by the Fischer exact test. As can be seen from Table 11, 50 percent of the litters which died in the high dose group had, for example, malaligned sternebrae compared with 20 percent in controls. The adequacy of these statistical evaluations appear questionable and perhaps should be reevaluated by OPP. However, even if the number of anomalies and variations were significant in the high dose group, the failure to find significant numbers of these effects in five litters examined in each of the controls and the lowest dose group may indicate that these effects did not occur below the highest dose level.

If comparable examinations were conducted in all F1a pups, a dose relationship may have been apparent in the anomalies and variations. There is no indication that this was done. A detailed study on developmental effects on the F1a pups which died during lactation was conducted but these numbers were insufficient to establish a NOEL. If the F1a pups were preserved, it may have been useful to have examined them for a dose related response in developmental effects. However, by day 28 of lactation, all of the apparent effects anologus to those seen in the F1b pups shortly after birth may have disappeared.

Dose levels consumed by dams around the perinatal period were greater for the F1b litters than for the F1a litters. The week immediately before parturition, gestational day 13-20, the dams of the F1a pups consumed the test substance at a daily rate of 76.1 mg/kg, while the dams of the F1b pup, during the corresponding time period consumed 107 mg/kg. The daily consumption of test substance by dams during the first week of lactation for the F1a and F1b pups was 112 and 133 mg/kg, respectively, in the 80 mg/kg target dose level group.

Table 11.

Total Number of Pups and Litters with Developmental and Genetic Variations - Only F1b Pups Found Dead Lactation Days 0-28

Dose Group	Pups 1	2	3	4	Litters 1	2	3	4
Number Examined Externally	5	15	3	103	5	5	3	18
Findings	----None----------				----None----------			
Number Examined Viscerally	5	15	3	103	5	5	3	18
Findings	----None----------				----None----------			
Number Examined Skeletally	5	14	3	98	5	5	3	18
Sternebra #5 and/or #6 Unossified	0	0	0	7	0	0	0	5
Sternebrae #1, #2, #3 and/or #4 Unossified	0	0	0	1	0	0	0	1
Sternebrae Malaligned (slight or moderate)	1	0	0	23	1	0	0	9
14th Rudimentary Rib(s)	0	0	0	12	0	0	0	6
Bent Rib(s)	0	0	0	30	0	0	0	6
Reduced Ossification of the Vertebral Arches	0	0	0	2	0	0	0	2

None significantly different from control group using Fisher's Exact Test.

7. Organ Weights and Histological Studies

The absolute and relative liver weights were reduced at all dose levels in the F0 males (table 12). Only the liver/body weight ratios are presented. The absolute and relative kidney weights were increased at all dose levels in F0 females (table 12). The report did not consider the effects on organ weights to be dose-related in either sex. No explanation for this opinion or for these possible test substance related effects was presented. However, neither effect exhibited a smooth dose-related decrease or increase, respectively.

In the F1 generation relative kidney weight of the left but not the right kidney was significantly elevated in males at the 20 mg/kg target dose level only (table 13). The relative liver weights in males of this group were apparently elevated but not statistically. The relative liver weights were increased in F1 females of this dose group but the apparently slightly elevated kidney weights, probably, are not dose related (table 13). Thus, the possible oragn weight effects in F1 generation failed to confirm the statistically significant organ weight effects seen in the F0 generation.

No organ weight effects or histopathology was seen in the testes from any dose level from any generation. No dose-related histological effects were seen in the ovary. Thyroids may have been saved but no histology was conducted on them. All the histological studies conducted failed to find any dose-related pathology in any of these organs in the F0 generation and the F1a, F1b, and F1 generation and F2a and F2b pups.

Two histological studies on the livers of the F0 animals were reported. One study was conducted by the testing facility (table 14), and the other was conducted by W. Ray Brown of Research Pathology Services,Inc., New Britain, P.A.(table 15).

When the livers from F0 males were examined histologically numbers of small foci of necrosis were found in all groups. This was initially diagnosed as Tyzzer's desease (table 14). This diagnosis was rejected because females were not affected, diarrhea was not detected, and survival was normal. Research Pathology Services found that small basophilic alterations in hepatocytes occurred at a slightly higher incidence in dosed animals(table 15). In females, these alterations occurred at a slightly higher incidence in controls. None of these histological findings were considered to be dose related by either pathologist.

Table 12.

F0 Terminal Body Weights and Relative Organ Weights

	Target Dose Levels mg/kg/day			
	0	5	20	80
F0 male bwt.	372.	373	368	354**
SD	15.4	17.8	18.3	19.5
F0 female bwt.	217	220	216	209**
SD	10.5	9.8	8.4	12.2
F0 male organ wt. per 100 g bwt.				
Lt Kidney	0.417	0.356**	0.421	0.435
SD	0.18	0.06	0.05	0.04
Rt Kidney	0.469	0.357**	0.420	0.429
SD	0.18	0.06	0.05	0.05
Liver	3.474	3.242**	3.337*	3.226**
SD	0.22	0.18	0.17	0.25
Testes	0.830	0.835	0.821	0.854
SD	0.04	0.04	0.05	0.08
F0 female organ wt. per 100 g bwt.				
Lt Kidney	0.351	0.471**	0.410*	0.425**
SD	0.14	0.12	0.05	0.04
Rt Kidney	0.361	0.476**	0.398	0.424
SD	0.15	0.11	0.06	0.04
Liver	3.477	3.663	3.608	3.627
SD	0.21	0.50	0.27	0.20

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$

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Table 13.

F1 Terminal Body Weights and Organ Weight Ratios

	Target Dose Levels[a] mg/kg/day		
	0	5	20
F1 male bwt.	394.	388	386
SD	13.8	28.8	22.9
F1 female bwt.	238	231	231*
SD	9.8	9.0	11.1
F1 male organ wt. per 100 g bwt.			
Lt Kidney	0.394	0.381	0.411*
SD	0.025	0.03	0.02
Rt Kidney	0.390	0.378	0.402
SD	0.02	0.03	0.02
Liver	3.315	3.345	3.439
SD	0.25	0.25	0.17
Testes	0.865	0.857	0.861
SD	0.06	0.11	0.08
F1 female organ wt. per 100 g bwt.			
Lt Kidney	0.398	0.406	0.419
SD	0.03	0.04	0.03
Rt Kidney	0.402	0.400	0.415
SD	0.03	0.05	0.03
Liver	3.568	3.566	3.808**
SD	0.27	0.33	0.25

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$
[a]F1 at 80 mg/kg/day target dose level not dosed beyond weaning.

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Table 15.

F0 histomorphological summary incidence
for liver, at terminal sacrifice.
Summary from Research Pathology Services

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number examined	30	30	30	30	29	29	30	29
Number normal	1	29	23	24	9	7	12	8
Multifocal bile duct proliferation	25	29	23	24	9	7	7	11
Focal necrosis	11	2	1	3	1	1	1	0
Multifocal necrosis	13	19	15	11	0	0	0	0
Focal cellular alteration								
Basophilic-cell focus/foci	0	4	3	6	8	9	3	1
Clear-cell focus/foci	1	0	0	2	0	0	0	0
Eosinophilic-cell focus/foci	0	0	0	0	0	0	1	0
Microgranuloma/s	2	5	4	2	6	3	7	9
Multifocal mononuclear cellular infiltration	6	2	4	3	6	10	6	8
Accessory lobe	1	1	0	3	2	1	0	3
Centrilobular hepatocelular vacuolation	0	0	0	1	0	0	0	0
Focal hepatocellular vacuolation	1	0	0	0	0	0	0	0
Congestion	0	0	0	1	0	0	0	0
Congenital anomaly	0	0	0	0	1	0	0	0

Table 14.

F0 histomorphological at terminal sacrifice.
Summary incidence for the live.
Testing laboratory summary.

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number of animals studied	30	30	30	30	29	29	30	29
Liver								
Total examined	30	30	30	30	29	29	30	29
Examined, unremarkable	6	3	9	13	20	22	20	17
Not examined	0	0	0	0	0	0	0	0
Cholangiofibrosis	21	20	19	14	5	3	5	5
Accessory lobe	1	1	0	3	2	1	0	3
Tyzzer's disease	4	18*	10	1	0	0	0	0
Nonspecific Kupffer cell granuloma	0	0	0	0	4	4	6	5

1= 0 mg/kg/day 2= 5 mg/kg/day 3= 20 mg/kg/day 4= 80 mg/kg/day

* Significantly different from control at 0.05 level, using Kolmogorov-Smirnov, one-tailed test.

8. Summary and Discussion

1) The study reviewed is a 2-generation, 2 litter per generation study of the effects of 2,4-dichlorophenoxy-acetic acid (2,4-D) on reproduction in Fischer 344 rats.

2) The test substance, (97.5% 2,4-D by an I.T.F analysis; and 95.8% 2,4-D by a WIL analysis) was administered in the feed, ad libitum, to 30 rats per sex per group. The concentration of the test substance was adjusted in the feed weekly or monthly according to food consumption and body weight in an attempt to meet target dose levels of 0, 5, 20 or 80 mg/kg/day. During gestation and lactation the actual dose level administered was generally higher, see table 1 and 2, even with 50 percent reduction in concentration during week 2 and 67 percent reduction in concentration during week 3 and 4 of lactation.

3) No significant effects on fertility of males or females at any dose or in any generation was evident. This conclusion is supported by the failure to find dose related effects on the testes weight or on histological examination of the testes. No dose related histological effects were seen in ovaries. There was no dose related differences in the number of second matings or in the time required for cohabitation. The fertility of Fischer 344 rats is not high, 60-79 percent in controls, and the variability of the fertility probably would allow detection of only severe reductions in fertility.

4) The lengths of gestation was prolonged by 1 day in approximately one half the F0 dams producing F1b litters only in the highest dose group. This effect could result from delayed implantation, hormonal imbalances, or parturition problems.

5) The mean body weights of the F0 generation were statistically significantly reduced compared to controls prior to mating, at the highest dose level. Since body weight gain per gram of food consumed was apparently nearly always less in the high dose group than in the other treatment groups or the controls, the body weight decrease cannot be explained by decreases in food consumption. At this dose level, food consumption was frequently satistically significantly

increased over control values. At the two lowest dose levels, food consumption was generally apparently increased, but it was seldom statistically significant. Thus, the weight reduction probably is real.

6) During lactation, the body weights of F0 dams in the high dose group were not consistently reduced and in the middle dose group in the F0 dams lactating for the F1b litters, there were no statistically significant reductions in body weight compared to controls. Note: It was in the mid dose group and during lactation for F1b litters, that the LEL for pup weight depression occurred.

7) The body weights of F1 females during gestation and lactation for F2a and F2b litters were infrequently significantly different from control weights(see table 6). After weaning of the F2b litters from week 44-77 were adult F1 female body weights significantly less than control weights for the target dose level of 20 mg/kg. The body weights of male F1 rats were not different from control weights at any time after weaning.

8) Pup body weights were significantly reduced over control weights in the F1a and F1b pups only. These reduced pup weights occurred at the highest dose throughout lactation and in the mid dose only toward the end of lactation, and only in the F1b pups. The NOEL was the lowest target dose level administered.

9) Pup viability was reduced at parturition and during the first day of lactation in F1a and F1b pups at the target dose level of 80 mg/kg (actual 76.1 to 133 mg/kg/day) only. A reduction in litter size probably also occurred in the highest dose group in the F1a litters. The apparent reduction probably was dominantly due to a decrease in number of female pups born, causing a significant difference in the sex ratio at birth.

Pup viability was more severely and significantly reduced in the F1b litters than in F1a litters at birth and between birth and lactation day 1 in addition to the period between lactation day 1 and lactation day 4. The sex ratio in these F1b pups was normal, probably because male, in addition to female pup viability, was less than in the F1a litters.

10) Anomalies and variations occurred in F1b litters of the high dose which died during lactation. This was the only group for which those effects could be determined because it was the only group apparently for which skeletal examinations were conducted. In addition, it was the only group in which a large number of nonscheduled pup deaths occurred.

These skeleltal anomalies and reductions in ossification are generally consistent with similar effects produced by 2,4-D in the teratogenicity study in Fischer 344 rats. The NOEL for developmental effects in that study is 25 mg/kg/day.

11) The absolute and relative liver weights of F0 males were statistically significantly reduced at all dose levels at terminal sacrifice. The absolute and relative kidney weights of F0 females were statistically elevated over control weights at all dose levels. There was not a "clean" dose-response relationship and the report did not consider the effect on either sex to be biologically significant.

The liver weight reductions seen in the males may not be toxicologically or pharmacologically significant, and could be an artifact of the study.
a) There was no "smooth" dose response relationship with the liver weight and the dose of the test chemical.
b) F1 males and females demonstrated no liver weight reductions.
c) No significant liver weight reductions occurred in a 90-day subchronic or a chronic study conducted at 1, 5, or 45 mg/kg/day in the Fischer 344 rat.
d) The reductions probably are not due to the slight thyroid effects analogus to the thyroid effects seen in the subchronic and chronic studies, because only higher elevations of T4 than those seen cause glycogen depletion in the liver.

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e)The reductions are not due to an interaction of 2,4-D with the liver histological findings seen. The liver weights in control animals with and without focal necrosis, multifocal necrosis, or basophilic alterations were each not different from each other. Similar comparisions failed to detect diffences in the highest dose level group.
f) Food consumption apparently increased at all the higher dose levels, and in some cases the increase was satistically significant. Thus, the liver weight reduction is not due a reduction in food consumption.

The satistically signiticant kidney weight increase in females of the F0 generation probably are not correlated with the kidney histopathology seen the males and female of the subchronic and chronic studies. No kidney histopathology was seen in any animals in the reproduction study. In addition, the kidney weights of 5 females in control animals were an average of 0.18 g for the left or the right kidney, whereas the average kidney weights in the remaining control animals were 0.9 g for the left or the right kidney, approximately 3 standard deviations different. Thus, if these 5 animals are removed from controls, the kidney weights in dosed animals are comparable to controls.

It is concluded that the kidney weight increase is due to an anomaly in the kidney weights of 5 control females, and that it is not due to the test substance.

12) No significant dose-related histopathology occurred in any organ at any dose level in any generation.

005446

References:

1. Subchronic toxicity study in Fischer 344 rats conducted by Hazleton Laboratories, Report No. 2184-102, dated September 12, 1983, for the Industry Task Force on 2,4-D Research No. 251474.
Feeding study conducted 90 days at dose levels of 0, 1, 5, 15, or 45 mg/kg/day.

2. Interim 52-week report on 2,4-D chronic feeding/ oncogenicity study in Fischer 344 rats. Conducted by Hazleton Laboratories submitted by the Industry Task Force on 2,4-D Research. Accession No. 256019.

Feeding study conducted at 0, 1, 5, 15, or 45 mg/kg/day.

3. Teratogenicity study of 2,4-D in Fischer 344 rats. Conducted at WIL Research Laboratories (WIL-81135) for the Industry Task Force on 2,4-D Research.

Study conducted at 0, 8, 25, or 75 mg/kg/day by gavage.

4. Reproduction study of 2,4-D in Fischer 344 rats. Conducted by WIL Research Laboratories (WIL-81137) for the Industry Task Force on 2,4-D Research. Accession No's. 259442-6.

CASWELL FILE

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005754

Subject: Addendum to the Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats: Correction on the Histopathology of the Kidneys of Males

From: David G Anderson, PhD.
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

To: Ms. Linda Vlier PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD.
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 1/14/87

An Addendum to the 2-generation rat reproduction feeding study on 2,4-D has been reviewed(accession # 265489). Only the LEL for the FO generation changed. The LEL now includes kidney pathology as well as body weight gain reduction. The recalulated and nominal NOEL's and the LEL's with their respective effects are as follows.

FO parental toxicity.
NOEL - 5(5) mg/kg/day.*
LEL - 20(20) mg/kg/day, male kidney pathology.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, male kidney pathology, and reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered). The values have been rounded off to the nearest whole number.

Reviewed by: David G Anderson David G Anderson 10/23/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 15, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day).

F0 parental toxicity
LEL- 19.9(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), kidney histopathology in males, and reduced body weight in females.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
0 (Control)	30	29	10
5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

Table 1.

Incidence of findings reexamination of kidneys from males from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	F0 adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.

005684

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(Sheet)

Subject: Addendum to the Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats: Correction on the Histopathology of the Kidneys of Males.

Caswell # 315

From: David G Anderson, PhD
Toxicology Branch
Section VII
Hazard Evaluation Division (TS-769C)

To: Ms. Linda Vlier, PM #61
Special Review Branch
Registration Division (TS-767C)

Thru: Albin B Kocialski, PhD.
Supervisory Pharacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

An Addendum to the 2-generation rat reproduction feeding study on 2,4-D has been reviewed(accession # 265089). The LEL and the NOEL in the F0 generation changed. The LEL is now 20 mg/kg/day, and the NOEL is now 5 mg/kg/day for the F0 generation. The LEL now includes kidney pathology as well as body weight reduction. However, the NOEL inclusive of all endpoints in the review of the original study is not altered by the results of this addendum. The recalulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 5(5) mg/kg/day.*
LEL - 20(20) mg/kg/day, male kidney tubule degeneration.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, male kidney tubule degeneration, and reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered). The values have been rounded off to the nearest whole number.

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005684

Reviewed by: David G Anderson David G. Anderson 10/23/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 1?, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day). The values have been rounded off to the nearest whole number.

F0 parental toxicity
LEL- 20(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), dengeration of male kidney tubules, and reduced body weight in females.
NOEL- 4(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

005684

A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
0 (Control)	30	29	10
5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

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C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

Table 1.

Incidence of findings reexamination of kidneys from males
from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	F0 adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.